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13. ABSTRACT *(Maximum 200 words)*

The primary objective of this research was to examine the feasibility of using immobilized-enzyme membranes to improve the performance and lifetime of a graywater filtration system. Enzyme-treated membranes were examined for increased flux relative to membranes without enzyme treatment. Of fifteen hydrolytic enzymes (seven proteases, six lipases, and two amylases) screened in soluble form for the ability to degrade synthetic graywater, Protease X from *Bacillus thermoproteolyticus rokko* showed the highest activity, and several enzymes (e.g., lipase Type II from porcine pancreas, and α-chymotrypsin from bovine pancreas) showed significant but lower activity. We also developed a new methodology for preparing biocatalytic films and paints based on polydimethylsiloxane (PDMS), which might be used as anti-fouling treatments for a wide variety of materials, including filters and membranes. Such enzyme treatments may increase the service lifetime and efficiency of filter membranes, resulting in dramatic cost savings and minimal maintenance for an eventual on-board graywater treatment system.

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FINAL REPORT

GRANT #: N00014-98-1-0696

PRINCIPAL INVESTIGATOR: Dr. Douglas S. Clark

INSTITUTION: University of California, Berkeley

GRANT TITLE: Performance-Enhancing Biomolecular Treatment Strategies for Naval Graywater Filtration Systems

AWARD PERIOD: 15 May 1998 - 31 December 2000

OBJECTIVE: To examine the feasibility of using immobilized-enzyme membranes to improve the performance and lifetime of a graywater filtration system.

APPROACH: Enzyme-treated membranes were examined for increased flux relative to membranes without enzyme treatment. As part of a three-way research project, two strategies were pursued at U.C. Berkeley: enzyme immobilization on the filter membrane surface via direct adsorption or covalent attachment, and enzyme immobilization via surface treatments with siloxane films. For example, polydimethylsiloxane, which is known to resist biological fouling, was used in the preparation of immobilized-enzyme silicone coatings for membrane-surface treatment. Once appropriate enzymes were identified, they were immobilized for testing against synthetic graywater.

ACCOMPLISHMENTS: We have designed and constructed a 96-channel filtration system for high throughput flux measurements with immobilized-enzyme membranes, enabling up to 96 enzymes and/or their combinations and immobilization methods to be screened simultaneously. Different enzymes and their combinations were screened for their ability to inhibit membrane fouling by graywater.

Of fifteen hydrolytic enzymes (seven proteases, six lipases, and two amylases) screened in soluble form for the ability to degrade synthetic graywater, Protease X from *Bacillus thermoproteolyticus rokko* showed the highest activity, and several enzymes (e.g., lipase Type II from porcine pancreas, and α-chymotrypsin from bovine pancreas) showed significant but lower activity. α-Chymotrypsin was then immobilized to PVDF ultrafiltration membranes

(molecular weight cutoff: 10,000) by seven different methods, with physical adsorption yielding the highest activity of immobilized enzyme for the hydrolysis of the model substrate benzoyl L-tyrosine ethyl ester. Based on this result, the enzymes were immobilized by physical adsorption to PVDF membranes, and the immobilized-enzyme membranes evaluated for flux improvement in filtration studies with synthetic graywater. As shown in Table I below, Protease X again had the greatest effect, producing a 70% improvement in flux over an 8-hour period.

Table I. Improvement of permeate fluxes by immobilized enzymes

Enzyme	Control	BSA	Trypsin	Chymotrypsin
Flux Gain (%)	0.0	0.6	9.3	38
Enzyme	Amylase *Bacillus* sp.	Amylase *B. licheniformis*	Lipase II (porcine)	Lipase AY30
Flux Gain (%)	0.7	6.5	49	16
Enzyme	Capalase (Lipase)	Lipase M	Lipase E.D.	Protease XXIII *A. oryzae*
Flux Gain (%)	6.9	14	43	23
Enzyme	Protease XIV *S. griseus*	Subtilisin Carlsberg	Italase (Lipase)	Protease K *T. album*
Flux Gain (%)	23	24	16	18
Enzyme	Protease X			
Flux Gain (%)	70			

In addition, a new type of ultrafiltration membrane, prepared from blended poly(vinylidene fluoride) (PVDF) and poly(MMA-co-MA) containing protease X (from *Bacillus thermoproteolyticus rokko*) was screened for antifouling activity against graywater. We also developed a new methodology for preparing biocatalytic films and paints

based on polydimethylsiloxane (PDMS), which might be used as anti-fouling treatments for a wide variety of materials, including filters and membranes.

To this end, the hydrolytic enzymes pronase and α-chymotrypsin were immobilized by either sol-gel entrapment or by a covalent attachment method into a polydimethylsiloxane (PDMS) matrix and cast into thin films or into an oil-based paint formulation. All of the coatings retained enzymatic activity and adhered to several different materials. PDMS immobilized enzyme also exhibited higher thermostability than enzyme in solution or covalently attached to the outer surface of the PDMS. A porous membrane based on PDMS-immobilized enzyme was also prepared by an immersion precipitation process. Protein adsorption measurements showed that the enzyme containing films and paints adsorbed less protein than enzyme-free controls, and that protein adsorption decreased with increasing proteolytic activity of the coating. These coatings thus provide the means to apply a stable enzymatic surface to a wide range of materials, and may be generally useful as biocatalytic paints with antifouling properties.

Finally, this research has also laid the foundation for follow-up research to develop a practical filtration system for shipboard application. This follow-up project is a tripartite collaboration between the polymer membrane synthesis and characterization laboratory of Professor Benny Freeman at N.C. State, the enzyme technology laboratory of Professor Douglas S. Clark at U.C. Berkeley, and the thin-film composite membrane development facility at Membrane Technology and Research, Inc. Furthermore, the biocatalytic methodology developed in this project could conceivably be extended to antifouling paints, coatings, and films for use on ship hulls, in implantable materials, and as protective coatings for medical devices.

SIGNIFICANCE: Effective methods for the treatment of shipboard wastewater are of considerable importance as the Navy moves forward in the 21st Century. The enzyme immobilization methods developed thus far are an important first step toward reducing filter fouling and increasing the operational lifetime of the filter membranes. Such enzyme treatments may increase the service lifetime and efficiency of filter membranes, resulting in dramatic cost savings and minimal maintenance for an eventual on-board graywater treatment system. Moreover, the biocatalytic

method we propose offers the Navy an environmentally friendly way of accomplishing this task. The ultimate products of this research will be biocatalytic filtration systems that resist fouling during the shipboard filtration of graywater. Such devices may generate interest for commercialization, and may be appropriate as a new technology for development by a start-up venture.

PUBLICATIONS AND PRESENTATIONS

1. Y.-D. Kim, J. S. Dordick, and D. S. Clark (2001), "Siloxane-Based Biocatalytic Films and Paints: New Enzyme-Containing Coatings with Foulant-Resistant Properties," Biotechnol. Bioeng., **72**, 475-482.

2. Y.-D. Kim, J. S. Dordick, and D. S. Clark, "Biocatalytic Polydimethylsiloxane Coatings," poster presented at Gordon Conference on Polymers (East), New London, CT, June, 2000.

3. Guo, Y. Yang, Y. D. Kim, and D. S. Clark, "High Throughput Screening of Hydrolytic Enzymes for Graywater Treatment and Prevention of Fouling," poster presented at ACS National Meeting, San Francisco, CA, March, 2000.